



Hematological, immunological and histological changes in guinea pigs in the treatment of microsporia with drugs “Micromar” and “Biogluk”

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Abstract

Microsporia is a common infectious disease in the practice of a veterinarian. This disease carries a risk of human infection because it is anthroponozoonotic. The main pathogen *Microsporum canis* affects the upper layers of the skin and has the ability to stay on the body of an animal for up to two years. Therefore, treatment should be carried out by a complex method and be aimed at preventing the spread of the pathogen in the environment and increase the body's resistance in the fight against the disease. “Micromar” based on clotrimazole and povidone iodine and the immunostimulant “Biogluk” based on beta glucan and biotin were used to treat patients with microsporia. Clotrimazole and povidone iodine have antifungal and antiseptic properties, and beta glucan in combination with biotin stimulates the immune response and accelerates the regeneration of damaged skin. In order to determine the immune reactivity of the organism in the treatment of microsporia, hematological, immunological blood tests and histological examinations of the skin of guinea pigs infected with the pathogen *M. canis* were performed. The obtained results showed that when using the antifungal drug “Micromar” and immunostimulant “Biogluk” the number of leukocytes decreases from 11.13 ± 0.72 to $6.95 \pm 0.10 \cdot 10^9/L$, rod-shaped neutrophils from 16.00 ± 1.29 to 6.17 ± 0.65 %, ESR from 5.67 ± 0.67 to 2.17 ± 0.31 mm/h, and the number of segmental neutrophils increases from 12.17 ± 1.47 to 22.00 ± 0.86 %. There is a tendency to increase T-helpers. These changes indicate the development of an active immune response to the inflammatory process. In the structure of the skin there is a decrease in exudation and infiltration in the dermis, active trophism of hair follicles, which indicates the course of regenerative processes.

Key words: guinea pig, microsporia, clotrimazole, povidone iodine, beta glucan, blood, leukocytes, lymphocytes, neutrophils, epidermis, dermis.

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1. Introduction

Microsporia is an infectious disease characterized by high contagiousness and the ability of the pathogen to persist on the body of the animal for up to two years. The most common pathogen of microspores is the fungus *Microsporum canis*. The disease is a danger to humans (Pototskyi, 2000; Medvedeva, 2002; Sharma et al., 2011). Therefore, in order to qualitatively treat microspores, a comprehensive approach using local and systemic antifungal drugs is needed. Therapeutic measures for microspores are aimed at healing the body and preventing the spread of the pathogen in the environment. However, the course of microsporia is often complicated by secondary microflora. Weakened functions of the immune system are a favorable factor that causes microsporia (Golovina, 1988; Sangoi et al., 2009; Iovenko & Koval, 2019).

For the treatment of microsporia we have developed drugs “Micromar” based on clotrimazole and povidone iodine and immunostimulant “Biogluk” based on beta-

glucan and biotin (Kisera et al., 2020). Clotrimazole and povidone iodine have antifungal and antiseptic properties, and beta-glucan in combination with biotin stimulates the immune response and accelerates the regeneration of damaged skin.

The purpose of the work is to conduct a study of the blood and skin of guinea pigs in the treatment of microsporia with the drugs “Micromar” and “Biogluk”.

2. Materials and methods

In order to determine the effectiveness of treatment of microsporia with drugs “Micromar” and “Biogluk”, studies were conducted on guinea pigs infected with microsporia. During treatment, blood tests and skin biopsies were performed at 7, 14 and 21 days after the start of the drugs to evaluate the results. Sick animals were given the drug “Micromar” once a day topically for 21 days and fed once a day the drug “Biogluk” for 21 days.

The research was conducted in the vivarium of Ternopil National Medical University named after I. Ya. Gorbachevsky. Hematological and immunological blood tests were performed in the laboratory of the Andriy Sheptytsky Metropolitan Hospital in Lviv. Histological examinations were performed in the laboratory of clinical and biological studies of State Scientific-Research Control Institute of veterinary drugs and feed additives.

The number of erythrocytes and leukocytes was determined by counting them in the Goryaev chamber, the hemoglobin content by the hemoglobin cyanide method.

The leukogram was derived on the basis of counting and differentiation of 200 leukocyte cells in blood smears stained by the method of Romanovsky-Gimza. This took into account cell size, size and shape of the nucleus, the presence and color of grains in the cytoplasm.

Hematocrit value was determined using hematocrit capillaries by centrifugation in a special centrifuge (10 minutes at 3000 rpm), erythrocyte sedimentation rate (for 1 hour) – using Panchenkov pipettes (Vlislo et al., 2012).

The T- and B-lymphocytes were counted using the erythrocyte diagnosticum of Ltd “Granum Laboratory”, Kharkiv. The principle of the method is based on the determination of subpopulations of T- and B-lymphocytes by the reaction of rosette formation with erythrocytes, on which adsorbed monoclonal antibodies against the receptors of CD3 (T-lymphocytes), CD4 (T-helpers), CD8 (T-suppressors), CD19 or (B-lymphocytes), CD16 (natural killers). The results of the study were recorded in a light microscope with an immersion system.

The results were statistically processed using the computer program Excel from the package “Microsoft Office 2007”. Probability was determined by t criterion. Material for histological examination (pieces of skin) was fixed in 10–12 % cooled solution of neutral formalin, followed by pouring in paraffin according to the scheme proposed by G. A. Merkulov (Merkulov, 1969). The fixed pieces with a thickness of 2 mm were washed in water for no less than 30 minutes, then dried on filter paper and passed through alcohols (75, 96 and 100° for dehydration and degreasing day in each of the alcohols). Then impregnated with a solvent of paraffin xylene for 1.5 hours. The xylene pieces were transferred to a saturated solution of paraffin in xylene

for 1.5 hours at a temperature of 37 °C. Then the pieces were kept in molten paraffin at a temperature of 54–55 °C, i.e. at a temperature 2–3° higher than the melting point of paraffin, up to 2 hours in the first portion of paraffin and up to 2 hours in the second. Then they were transferred to porcelain cups and filled with molten paraffin, followed by rapid cooling in a refrigerator. After the paraffin has hardened, the pieces are cut with paraffin and glued to wooden cubes. Histosections with a thickness of 5–7 µm were made on a sled microtome MS-2. Hematoxylin and eosin staining were used for morphological evaluation of cells and tissues. Microscopy was performed using an OLIMPUS CX-41 microscope. The experiment complies with ARRIVE rules. In addition, it was carried out in accordance with the Animal Act in Great Britain (Scientific Procedures) 1986 and the relevant instructions.

Data analysis was performed using Statistica 6.0 (StatSoft Inc., USA). The data are presented in the tables as $x \pm SD$ ($x \pm$ standard deviation). Differences between values in the control and experimental groups were determined using ANOVA, where the differences were considered significant at $P < 0.05$ (taking into account the Bonferroni error).

3. Results and discussion

Hematological studies showed that in the treatment of microsporia using the antifungal drug “Micromar” and immunostimulant “Biogluk” there is a probable decrease in white blood cells from 11.13 ± 0.72 to 6.95 ± 0.15 G/L, rod neutrophils from $15, 76 \pm 1.29$ to 6.17 ± 0.65 %, ESR from 5.67 ± 0.67 to 2.17 ± 0.31 mm/h and a probable increase in segmental neutrophils from 12.17 ± 1.47 to 22.00 ± 0.86 %, (Table 1). From the studied immunological parameters (table 2) we noted a gradual increase in T-helpers on the 7th, 14th and 21st day of treatment. The content of T-helpers according to the literature increases with the body's response to the inflammatory process and characterizes the resistance status of the immune system in the fight against the disease (Scott et al., 2001; Sangoi et al., 2009). There is an active immune response to inflammation of infectious etiology (Kondrakhin et al., 2004).

Table 1

Morphological parameters of blood in guinea pigs in the treatment of microsporia with drugs “Micromar” and “Biogluk” ($M \pm m$, $n = 6$)

Index	Units of measurement	sick	Treatment		
			Day 7	Day 14	Day 21
Hematocrit	%	37.17 ± 7.6	36.83 ± 1.17	36.83 ± 0.83	35.83 ± 0.60
Hemoglobin	g/L	113.33 ± 4.51	115.00 ± 3.81	115.83 ± 4.17	120.00 ± 3.44
Erythrocytes	$10^{12}/L$	5.25 ± 0.21	5.73 ± 0.11	5.84 ± 0.08	5.82 ± 0.15
Platelets	g/L	231.17 ± 7.60	206.00 ± 5.16	213.50 ± 5.99	218.67 ± 6.95
Leukocytes	$10^9/L$	11.13 ± 0.72	9.57 ± 0.45	$7.35 \pm 0.28^{**}$	$6.95 \pm 0.15^{**}$
Neutrophils	R	%	15.76 ± 1.29	12.33 ± 0.80	$8.83 \pm 0.60^{***}$
	S	%	12.17 ± 1.47	$19.01 \pm 1.65^{**}$	$21.33 \pm 1.56^{***}$
Eosinophils	%	2.70 ± 0.73	2.83 ± 0.54	2.83 ± 0.79	3.17 ± 0.48
Basophils	%	0	0	0	0
Monocytes	%	4.17 ± 0.83	6.00 ± 0.58	5.18 ± 0.65	5.17 ± 0.75
Lymphocytes	%	65.20 ± 1.48	59.83 ± 1.04	61.83 ± 0.79	63.50 ± 0.67
ESR	mm/hour	5.67 ± 0.67	3.67 ± 0.42	$2.00 \pm 0.37^{**}$	$2.17 \pm 0.31^{**}$
Color indicator		0.76 ± 0.02	0.78 ± 0.01	0.75 ± 0.01	0.76 ± 1.01

Note: probability of differences with clinically healthy animals: * – at $P < 0.05$; ** – at $P < 0.01$; *** – at $P < 0.001$

Table 2

Immunological parameters of the blood of guinea pigs in the treatment of microsporia with the drugs “Micromar” and “Biogluk” ($M \pm m$, $n = 6$)

Index	Units of measurement	sick	Treatment		
			Day 7	Day 14	Day 21
B-lymphocytes	%	22.33 ± 0.95	24.67 ± 0.88	26.33 ± 1.05	24.00 ± 0.58
T-lymphocytes	%	53.83 ± 0.85	53.00 ± 0.89	50.33 ± 0.84	51.83 ± 1.01
0-lymphocytes	%	23.84 ± 1.14	22.33 ± 1.12	23.34 ± 1.36	24.17 ± 1.35
Natural killers	%	19.33 ± 0.84	19.00 ± 1.39	18.83 ± 0.79	17.50 ± 1.18
T- helpers	%	33.33 ± 0.71	34.33 ± 0.67	36.50 ± 0.76	37.33 ± 0.49
T- suppressors	%	23.00 ± 1.51	22.17 ± 0.79	19.33 ± 0.49	21.00 ± 0.68

The results of histological examination of the skin on the 7th day of treatment are characterized by clear visualization of the layers of the epidermis (Fig. 1). There is no thickening of the stratum corneum and hyperplasia of the basal layers of the epidermis, which arise due to the prolonged course of microspores (Medvedeva et al., 2002; Makurina, 2016). In the dermis, dilation of blood capillaries and slight weakening are noted, which may indicate residual inflammatory reactions, but not inflammatory infiltration (Cooke, 2012).

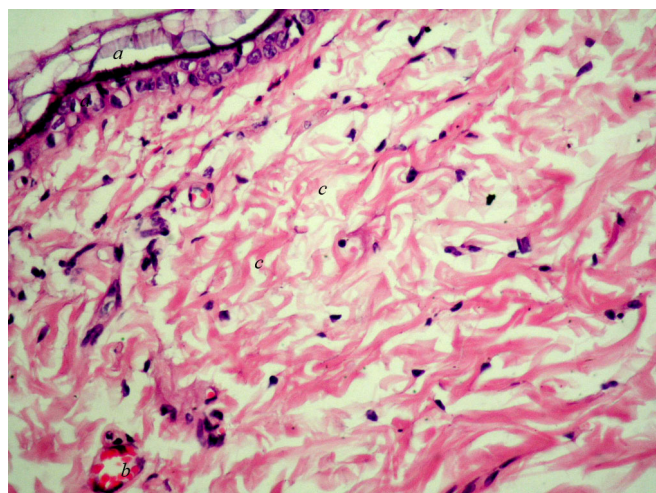


Fig. 1. Histological structure of guinea pig skin on the 7th day of treatment: a – epidermis; b – dilation of blood capillaries; c – derma. Hematoxylin and eosin. EG. 10, vol. 10

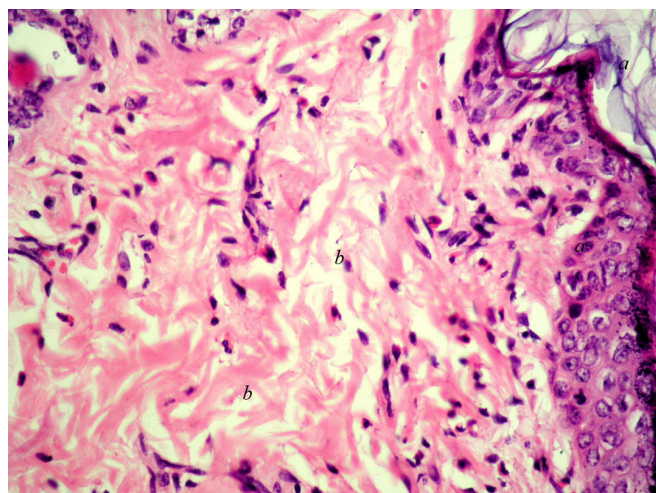


Fig. 2. Histological structure of guinea pig skin on the 14th day of treatment: a – epidermis; b – dermis (single erythrocytes in the thickness). Hematoxylin and eosin. EG. 10, vol. 20

On day 14, there was a clear differentiation of the layers of the epidermis without signs of inflammation and dystrophic changes (Fig. 2). There are single erythrocytes in the dermis. These changes characterize the resolution of the inflammatory process in the skin (Medvedeva et al., 2002; Makurina, 2016).

On day 21, the external root sheath of hair follicles is visualized (Fig. 3), which indicates the restoration of hair via activation of trophic processes in the structure of the epithelium of hair follicles, which is consistent with the literature (Scott et al., 2001; Cooke, 2012).

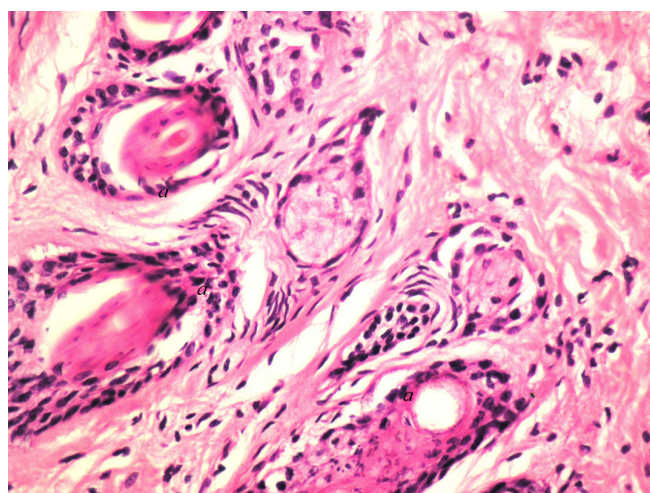


Fig. 3. Histological structure of guinea pig skin on the 21st day of treatment: a-epithelium of hair follicles. Hematoxylin and eosin. EG. 10, vol. 20

4. Conclusions

Treatment with drugs “Micromar” and “Biogluk” is accompanied by a decrease in the number of leukocytes, rod-shaped neutrophils and ESR, an increase in the number of segmental neutrophils.

The increase in T-helpers during treatment characterizes the body's protective response and its resistance status.

When using drugs “Micromar” and “Biogluk” in the skin there are regenerative processes and active hair trophism.

Prospects for further research. To test the developed drugs “Micromar” and “Biogluk” on cats with microsporida.

Conflict of interest

The authors declare that there is no conflict of interest.

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